

Structures Immunoreactive with Porcine NPY in the Caudal Neurosecretory System of Several Fishes and Cyclostomes

Authors: Oka, Shunya, Chiba, Akira, and Honma, Yoshiharu

Source: Zoological Science, 14(4) : 665-669

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.14.665>

The BioOne Digital Library (<https://bioone.org/>) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (<https://bioone.org/subscribe>), the BioOne Complete Archive (<https://bioone.org/archive>), and the BioOne eBooks program offerings ESA eBook Collection (<https://bioone.org/esa-ebooks>) and CSIRO Publishing BioSelect Collection (<https://bioone.org/csiro-ebooks>).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Structures Immunoreactive with Porcine NPY in the Caudal Neurosecretory System of Several Fishes and Cyclostomes

Shunya Oka^{1*}, Akira Chiba¹ and Yoshiharu Honma²

¹*Department of Biology, School of Dentistry at Niigata, Nippon Dental University, Niigata 951, Japan*

²*Department of Anatomy, Niigata University School of Medicine, Niigata 951, Japan*

ABSTRACT—Using a double immunostaining technique, we examined the relationships between neuropeptide Y (NPY)- and urotensin I (UI)- and/or urotensin II (UII)-positive structures in the caudal spinal cord of various species of fishes, particularly in the teleost *Oncorhynchus masou* and in elasmobranchs *Scylliorhinus torazame*, *Triakis scyllium*, and *Raja kenoei*. Primitive actinopterygians, i.e., *Acipenser transmontanus*, *Polypterus senegalus*, and *Lepisosteus productus*, and cyclostomes *Lethenteron japonica* and *Eptatretus atami* were also studied from the viewpoint of comparative endocrinology. In the teleost and elasmobranchs, NPY-positive fibers were demonstrated to be often in contact with UI- and/or UII-positive cells and fibers, suggesting a control of the caudal neurosecretory neurons by NPY or a related substance. Similar association was seen in the primitive actinopterygians. As to the cyclostomes, immunoreactivities for NPY and UII were found in the lamprey, but none of the immunoreactivity for these peptides was seen in the hagfish. In the lamprey, NPY-positive varicose fibers abutting on the UII-positive cells were also demonstrated.

INTRODUCTION

The caudal neurosecretory system designated by Enami (1955) consists of the neurosecretory cells, fibers, and neurohemal terminal complex, the urophysis. In spite of great structural variations, this system is generally found in the caudal spinal cords of bony fishes and cartilaginous fishes. However, it is exceptionally absent in dipnoans and holocephalans (Kobayashi *et al.*, 1986). On the other hand, in cyclostomes and cephalochordates, no structural elements of the caudal neurosecretory system were demonstrated (Sano, 1965; Kobayashi *et al.*, 1986).

Two major peptide hormones, urotensin I (UI) and urotensin II (UII), have been well characterized chemically and pharmacologically. UI has an amino acid sequence homologous with corticotropin-releasing factor (CRF) and sauvagine (Lederis *et al.*, 1982; Ichikawa *et al.*, 1982), whereas UII is partially homologous with somatostatin (Pearson *et al.*, 1980; Ichikawa *et al.*, 1984). Subsequent immunohistochemical studies using antisera raised against UI and UII demonstrated cellular and subcellular localization of these peptides in various fish species (Kobayashi *et al.*, 1986; Larson *et al.*, 1987; Oka *et al.*, 1989). In addition, the presence of UII-immunoreactive cerebrospinal fluid (CSF)-contacting neurons have

been shown in the extraurophyseal region of the spinal cord (Yulis and Lederis, 1986, 1988; Oka *et al.*, 1989). It was also demonstrated, by *in situ* hybridization studies, that all caudal neurosecretory cells in the carp are able to synthesize both UI and UII (Ichikawa *et al.*, 1988). However, the manner in which differential synthesis and release are regulated remains unknown (Bern, 1990).

Concerning the control of the caudal neurosecretory system, many studies have shown that the neurosecretory cells receive synaptic contacts (Kobayashi *et al.*, 1986) and that the cells do not operate autonomously (O'Brien and Kriebel, 1982). The chemical nature of the neuronal input to the caudal neurosecretory system has been analyzed by various techniques including fluorescence histochemistry (Audet and Chevalier, 1981), electron microscopy (O'Brien and Kriebel, 1983; Miller and Kriebel, 1986a), and immunohistochemistry (Miller and Kriebel, 1986b; Onstott and Elde, 1986; Yulis *et al.*, 1990), and was demonstrated to be cholinergic, catecholaminergic (Kriebel *et al.*, 1985), serotonergic (Onstott and Elde, 1986; Yulis *et al.*, 1990), and peptidergic (Miller and Kriebel, 1986b).

Brain centers that project to the caudal neurosecretory perikarya have also been demonstrated (Kriebel *et al.*, 1985). However, information on the peptidergic innervation of the caudal neurosecretory system is too meager to evaluate its contribution to the control mechanisms; one paper (Miller and Kriebel, 1986b) reported projection of gonadotropin-releasing

* Corresponding author: Tel. +81-25-267-1500 ext. 529; FAX. +81-25-267-1134.

hormone (GnRH)-immunoreactive fibers to the caudal neurosecretory system in a cyprinodont fish.

In light of the scanty information on peptidergic innervation, the present immunohistochemical study was designed to elucidate whether or not neuropeptide Y (NPY)-containing fibers might innervate the caudal neurosecretory system of various fish groups, mainly teleost and elasmobranchs, because NPY is known to be a regulatory peptide that occurs widely and abundantly in the central nervous system of higher vertebrates and to act as a neurotransmitter or neuromodulator (Colmers and Whalestedt, 1993). Our previous data on fish brains have supported this view (Chiba and Honma, 1992, 1994; Chiba and Oka, 1996; Chiba *et al.*, 1993).

MATERIALS AND METHODS

Four species of Osteichthyes, the yamame, *Oncorhynchus masou*; the Senegal bichir, *Polypterus senegalus*; the spotted gar, *Lepisosteus productus*; and the white sturgeon, *Acipenser transmontanus*, three species of Chondrichthyes, the banded dogfish, *Triakis scyllium*; the cloudy dogfish, *Scyliorhinus torazame*; and the thornback ray, *Raja kenoei*, two species of Agnatha, the lamprey *Lethenteron japonica* and the hagfish *Eptatretus atami* were used in this study (Table 1). Except for the Senegal bichir, the spotted gar, and the white sturgeon, which were obtained through a local dealer, the animals were collected from the coastal and inland waters of Niigata Prefecture. After having been anesthetized with MS-222, the animals were perfused through the heart with acid-free Bouin's fluid. Then the caudal spinal cords were dissected out, immersed in the same fixative, dehydrated with graded ethanols, and embedded in paraffin. Serial cross and sagittal sections were cut at a 10- μ m thickness, mounted on gelatin-coated glass slides, and immunostained by the streptavidin-biotin method with a commercial kit (Nichirei, Tokyo). The following antisera at the specified dilution were used as the primary antibodies: anti-porcine NPY (1:3000; UCB Bioproducts, Belgium), anti-*Catostomus* UI (1:4000; Suess *et al.*, 1986), and anti-*Gillichthys* UII (1:3000; Bern *et al.*, 1985). For simultaneous demonstration of NPY and UI/UII immunoreactivities in the same section, double immunoenzymatic labeling was applied according to the rec-

ommendation of Nakane (1968) and Hsu and Soban (1982). Controls were prepared by replacement of the specific antiserum with (1) normal rabbit serum, (2) antiserum preabsorbed with homologous antigens, i.e., synthetic UI, UII (1 μ M; Sigma Chemical Company, USA), and NPY (1 μ M; Peninsula Laboratories, USA), or (3) antiserum pretreated with related peptides, i.e., human CRF (10 μ M; Peptide Institute Inc, Japan), somatostatin-14 (10 μ M; Funakoshi, Japan), synthetic avian pancreatic polypeptide (10 μ M, Peninsula Lab.) and peptide YY (10 μ M, Peninsula Lab.). Immunostaining was negative in all sections treated with the control procedures (1) and (2), but was not abolished in those treated with procedure (3).

RESULTS

NPY-like-immunoreactive fibers were demonstrated in the caudal spinal cord of most of the species examined, with the exception of the hagfish. The frequency and density of the fibers showed variations among the different species. The results of NPY immunohistochemistry are summarized in Table 1 together with those of UI and UII immunohistochemistry.

Osteichthyes

Sagittal sections of the caudalmost eight segments of the spinal cord of the yamame revealed fine-beaded fibers showing moderate or intense NPY immunoreactivity. No NPY-positive cells were found in this area, but the NPY-positive fibers were scattered throughout the entire spinal cord. In the median sections, some NPY-positive fibers were intermingled with UI- and/or UII-positive cells and their processes (Fig. 1). Occasionally, NPY-positive fibers approached closely and appeared to come in contact with UI/UII-positive fibers, but the formers were never found in the neurohemal terminal area. In the caudal spinal cords of the spotted gar and the white sturgeon, the distributional outline of NPY and UI/UII immunoreactivities was similar to that of the yamame. The Senegal bichir showed interesting features in the distribution of UI- or UII-

Table 1. Occurrence of NPY-positive (NPY+ve) fibers in the spinal cord of the animals examined and contact with urotensin-positive (U+ve) structures

Name of animals	Age	NPY+ve fibers	Contact with U+ve cells	Contact with U+ve fibers
Osteichthyes				
<i>Oncorhynchus masou</i>	Juv	+	UI/UII	UI/UII
<i>Polypterus senegalus</i>	Ad	++	UI or UII	UI or UII
<i>Lepisosteus productus</i>	Ad	+	UI/UII	UI/UII
<i>Acipenser transmontanus</i>	Juv	+	UI/UII	UI/UII
Chondrichthyes				
<i>Triakis scyllium</i>	Juv	++	UI/UII	UI/UII
<i>Scyliorhinus torazame</i>	Ad, Juv	++	UI/UII	UI/UII
<i>Raja kenoei</i>	Ad, Juv	++	UI/UII	UI/UII
Agnatha				
<i>Lethenteron japonica</i>	Ad	+	UII	UII
<i>Eptatretus atami</i>	Ad	-	-	-

Ad, adult; Juv, juvenile.

Score: ++, frequent; +, less frequent; -, not detected.

UI/UII: contact with UI and/or UII-positive structures.

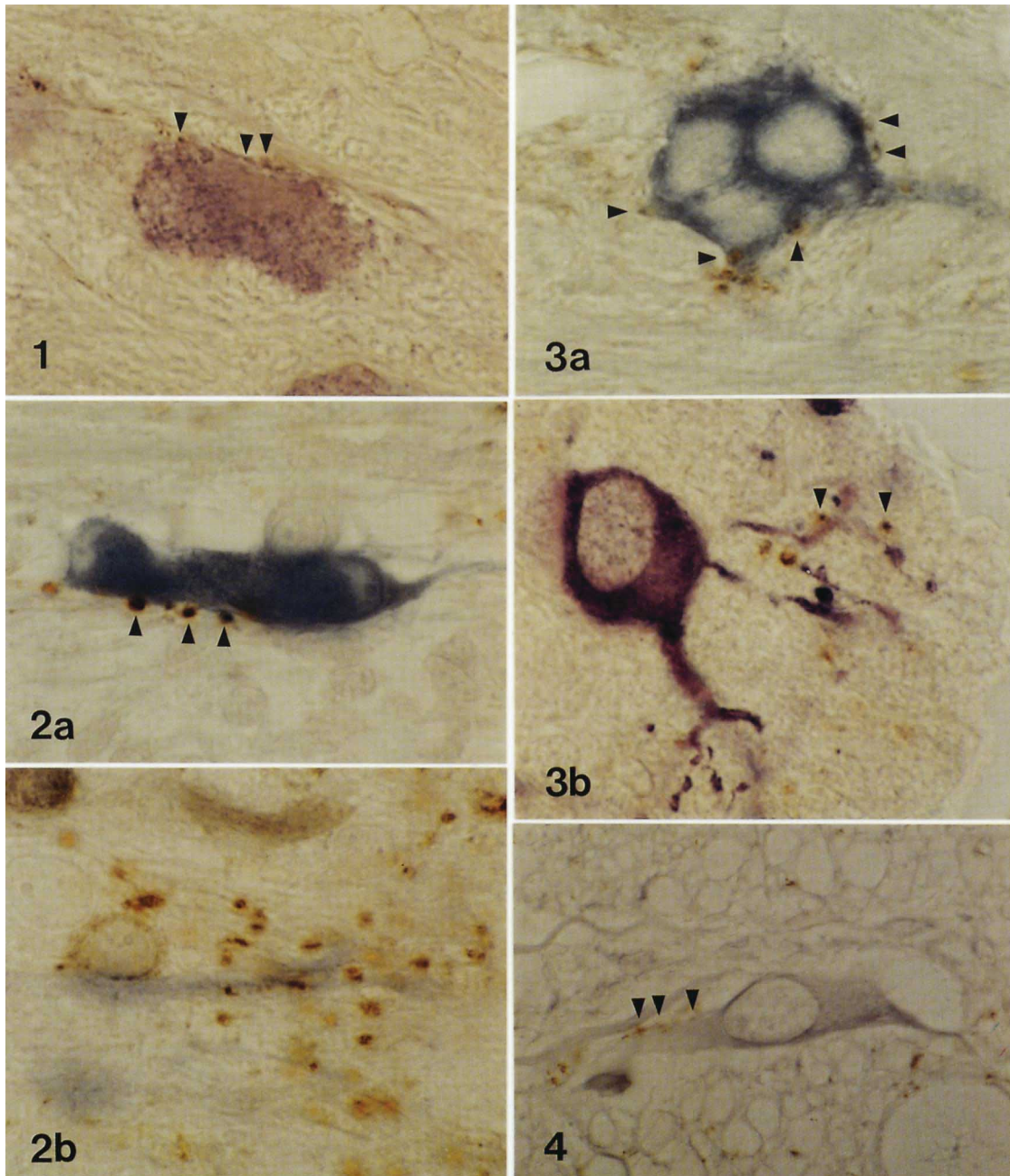


Fig. 1. Double immunohistochemical technique applied to the caudal spinal cord of the yamame, *Oncorhynchus masou*, showing close appositions of NPY-positive fibers (arrowheads: brown elements) to a UI-positive cell (Purple element). $\times 1000$.

Fig. 2a. Double immunohistochemical technique applied to the anterior part of the spinal cord near the medulla oblongata of the bichir, *Polypterus senegalus*, showing contact of NPY-positive fibers (arrowheads: brown elements) with a UI-immunoreactive cell (dark blue element). $\times 1000$.

Fig. 2b. Close relationship between NPY-positive cells and fibers (brown elements) and UI-positive fibers (dark blue elements) in the same specimen shown in Fig. 2a. $\times 1000$.

Fig. 3a. Simultaneous demonstration of UI- and NPY-positive structures in the caudal spinal cord of the banded dogfish, *Triakis scyllium*. Note NPY-positive fibers (arrowheads: brown elements) abutting on a UI-positive cell (dark blue element). $\times 1000$.

Fig. 3b. Double immunohistochemical technique applied to the caudal spinal cord of the dogfish, *Scyliorhinus torazame*, showing NPY-positive fibers (arrowheads: brown elements) in contact with UI-positive fibers (purple elements). $\times 1000$.

Fig. 4. Caudal spinal cord of the lamprey, *Lethenteron japonica*, showing close apposition of UI-positive cell (grayish blue) and NPY-positive fibers (arrowheads: brown elements). $\times 1000$.

cells and fibers; even in the anterior part of spinal cord near the medulla oblongata, UI-positive cells were often observed. There NPY-positive varicose fibers were often demonstrated

to be abutting on the UI-positive cells (Fig. 2a). In addition, NPY-positive cells and fibers were often observed to be intermingled with UI-immunoreactive cells and fibers (Fig. 2b).

Contacts of UI- and NPY-positive structures were seen widely in the entire spinal cord except for the caudalmost segments. In the bichir, UII-positive cells and fibers were seen in a limited area, i.e., the caudalmost part of the spinal cord, where they were found in contact with NPY-positive fibers.

Chondrichthyes

In the banded dogfish, UI/UII-positive cells with a large lobulated nucleus were aligned laterally to the central canal. On the other hand, NPY fibers were found mainly in the dorsal area of the spinal cord; but some of the fibers coursed ventrally and tended to be concentrated in the areas ventrolateral to the central canal, where the UI/UII-immunoreactive neurosecretory cells were located. Contacts of NPY-positive fibers with the UI/UII-positive cells and processes were often observed (Fig. 3a). Nearly the same pattern was seen in the cloudy dogfish (Fig. 3b) and the thornback ray. Noticeably, in the ray, NPY-positive cells were rarely observed near the caudal neurosecretory cells, although NPY-positive processes were not traced. In the sharks and ray, as well as in the bony fishes, no NPY-positive fibers were seen in the neurohemal terminal area of the caudal neurosecretory system.

Agnatha

In the caudal spinal cord of the lamprey, UII-positive cells and fibers were barely recognized. NPY-positive fibers were seen in contact with the UII-positive structures (Fig. 4). On the other hand, in the hagfish, neither NPY- nor UI/UII-positive structures were detected in any spinal cord segment.

The present study gave no evidence for colocalization of NPY and urotensins at somal and fiber levels in any specimen examined.

DISCUSSION

The present study demonstrated that NPY-positive fibers are often in contact with UI/UII-positive cells and processes in most of the species that belong to representative groups of fishes, with the exception of the negative result for the hagfish. NPY is generally considered to be an essential peptidergic neurotransmitter (Colmers and Whalestedt, 1993), and its primary structure is highly conserved and its cDNA libraries of the goldfish and the ray, *Torpedo marmorata*, show strong conservation between the data of mammals (Blomqvist *et al.*, 1992). Moreover, NPY-related peptide has been isolated from the central nervous system of the lamprey (Rawitch *et al.*, 1992). In the light of such data, it is natural to consider that NPY or some related substance in the immunostained fibers may also serve as transmitter or modulator in the caudal neurosecretory system. However, further studies should be carried out to determine chemical nature of antigens that were demonstrated immunohistochemically in the present study.

Noteworthy, UII-positive cells were detected in the lamprey, and these cells were also in contact with NPY-positive fibers. It has been generally accepted that cyclostomes do not possess any elements of the caudal neurosecretory sys-

tem, based on not only routine histological studies (Sano, 1965) but also UI/UII immunohistochemistry (Owada *et al.*, 1985). However, UI/CRF-immunopositive fibers were detected in the caudal spinal cord of the larval sea lamprey, *Petromyzon marinus* (Onstott and Elde, 1986). Moreover, UII was recently isolated from the brains of two species of lampreys (Waugh *et al.*, 1995). Thus, reinvestigation of the caudal neurosecretory system of lampreys would appear to be in order. In the hagfish, no NPY immunoreactivity was detected in the caudal spinal cord segments, although NPY-positive cells and fibers were demonstrated in various brain areas (Chiba *et al.*, 1993). Neither UI nor UII immunoreactivity was detected in the hagfish specimens. This result is consistent with earlier immunohistochemical observations on *Eptatretus stouti* (Onstott and Elde, 1986). However, it cannot be excluded that the hagfish contain unknown related substances that are structurally and/or immunologically different from urotensins of lampreys and fishes.

In the caudal neurosecretory system of the molly, *Poecilia sphenops*, it was reported that descending peptidergic innervation may arise from the dorsal tegmental magnocellular nucleus (Miller and Kriebel, 1986a) and that some neurons of the nucleus show GnRH immunoreactivity (Miller and Kriebel, 1986b). Moreover, at least some of the peptidergic terminals appear to arise from collaterals of the caudal neurosecretory system of the same species (O'Brien and Kriebel, 1983). According to previous mapping studies on fish brains, the distribution of NPY immunoreactivity in the dorsal tegmentum differed from species to species (Danger *et al.*, 1991; Vecino and Ekström, 1992; Chiba and Honma, 1992, 1994; Chiba and Oka, 1996). Additional experiments are required to determine the origin of NPY-positive fibers and to elucidate the functional significance of NPY or related substances in the caudal neurosecretory system.

In addition to NPY-positive fibers, in an earlier study (Oka *et al.*, 1993) we detected substance P- and FMRFamide (molluscan cardioexcitatory tetrapeptide)-positive fibers in contact with UI/UII-positive cells in the caudal neurosecretory system of some of the fishes examined in the present study. These data suggest a possibility of multipeptidergic regulation of caudal neurosecretory functions.

ACKNOWLEDGMENTS

We are much indebted to Emeritus Professor Dr. K. Lederis, University of Calgary, Canada, for kindly supplying antisera against both urotensins through Prof. Dr. T. Fujita, Nippon Dental University, Niigata, and to Prof. Dr. Iwanaga, Hokkaido University, Sapporo. We thank Mr. Yukihiko Nakamura, Director of Johetsu Municipal Aquarium, for supplying some of the fish specimens.

REFERENCES

- Audet C, Chevalier G (1981) Monoaminergic innervation of the caudal neurosecretory system of the brook trout *Salvelinus fontinalis* in relation to osmotic stimulation. *Gen Comp Endocrinol* 45: 189–203
- Bern HA (1990) The caudal neurosecretory system: quest and be

- quest. In "Progress in Comparative Endocrinology" Wiley-Liss, Inc, pp 242–249
- Bern HA, Pearson D, Larson BA, Nishioka RS (1985) Neurohormones from fish tails: the caudal neurosecretory system. I. "Urophysiology" and the caudal neurosecretory system of fishes. *Recent Prog Horm Res* 41: 533–552
- Blomqvist AG, Soderberg C, Lundell I, Milner R, Larhammar D (1992) Strong evolutionary conservation of neuropeptide Y: sequence of chicken, goldfish, and *Torpedo marmorata* DNA clones. *Proc Natl Acad Sci USA* 89: 2350–2354
- Chiba A, Honma Y (1992) Distribution of neuropeptide Y-like immunoreactivity in the brain and hypophysis of the cloudy dogfish, *Scyliorhinus torazame*. *Cell Tissue Res* 268: 453–461
- Chiba A, Honma Y (1994) Neuropeptide Y-immunoreactive structures in the telencephalon and diencephalon of the white sturgeon, *Acipenser transmontanus*, with special regard to the hypothalamo-hypophyseal system. *Arch Histol Cytol* 57: 77–86
- Chiba A, Oka S (1996) Distribution of neuropeptide Y-like immunoreactivity in the brain and hypophysis of the primitive bony fish, *Polypterus senegalus*. *Zool Sci* 13 (Suppl): 21
- Chiba A, Honma Y, Oka S (1993) Immunohistochemical localization of neuropeptide Y-like substance in the brain and hypophysis of the brown hagfish, *Paramyxine atami*. *Cell Tissue Res* 271: 289–295
- Colmers WF, Whalestedt C (1993) The Biology of Neuropeptide Y and Related Peptides. Humana Press, Totawa, New Jersey
- Danger JM, Breton B, Vallarino M, Fournier A, Pelletier G, Vaudry H (1991) Neuropeptide-Y in the trout brain and pituitary: localization, characterization, and action on gonadotropin release. *Endocrinology* 128: 2360–2368
- Enami M (1955) Studies in neurosecretion. II. Caudal neurosecretory system in the eel (*Anguilla japonica*). *Gunma J Med Sci* 4: 23–36
- Hsu SM, Soban E (1982) Color modification of diaminobenzidine (DAB) precipitation by metallic irons and its application for double immunohistochemistry. *J Histochem Cytochem* 30: 1079–1082
- Ichikawa T, McMaster D, Lederis K, Kobayashi H (1982) Isolation and amino acid sequence of urotensin I, a vasoactive and ACTH-releasing neuropeptide, from the carp (*Cyprinus carpio*) urophysis. *Peptides* 3: 859–867
- Ichikawa T, Lederis K, Kobayashi H (1984) Primary structures of multiple forms of urotensin II in the urophysis of the carp, *Cyprinus carpio*. *Gen Comp Endocrinol* 55: 133–141
- Ichikawa T, Ishida I, Ohsako S, Deguchi T (1988) *In situ* hybridization demonstrating coexpression of urotensins I, II- α , and II- γ in the caudal neurosecretory neurons of the carp *Cyprinus carpio*. *Gen Comp Endocrinol* 71: 493–501
- Kobayashi H, Owada K, Yamada C, Okawara Y (1986) The caudal neurosecretory system in fishes. In "Vertebrate Endocrinology" Ed by Pang PKT, Schreibman MP, Academic Press, New York, London, pp 147–174
- Kriebel RM, Parsons RL, Miller KE (1985) Innervation of caudal neurosecretory cells. In "Neurosecretion and the Biology of Neuropeptides" Ed by Kobayashi H, Bern HA, Urano A, Jpn Sci Soc Press, Tokyo, pp 205–211
- Larson BA, Bern HA, Lin RJ, Nishioka RS (1987) A double sequential immunofluorescence method demonstrating the colocalization of urotensins I and II in the caudal neurosecretory system of the teleost, *Gillichthys mirabilis*. *Cell Tissue Res* 247: 233–239
- Lederis K, Letter A, McMaster D, Moore G, Schlesinger D (1982) Complete amino acid sequence of urotensin I, a hypotensive and corticotropin-releasing neuropeptide from *Catostomus commersoni*. *Science* 218: 162–164
- Miller KE, Kriebel RM (1986a) Cytology of brain stem neurons projecting to the caudal neurosecretory complex: An HRP-electron microscopic study. *Brain Res Bull* 16: 183–188
- Miller KE, Kriebel RM (1986b) Peptidergic innervation of caudal neurosecretory system. *Gen Comp Endocrinol* 64: 396–400
- Nakane PK (1968) Simultaneous localization of multiple tissue antigens using the peroxidase-labeled antibody method; a study on pituitary glands of the rat. *J Histochem Cytochem* 16: 557
- O'Brien JP, Kriebel RM (1982) Brain stem innervation of the caudal neurosecretory system. *Cell Tissue Res* 227: 153–160
- O'Brien JP, Kriebel RM (1983) Caudal neurosecretory system synaptic morphology following deafferentation: An electron microscopic degeneration study. *Brain Res Bull* 10: 89–95
- Oka S, Honma Y, Iwanaga T, Fujita T (1989) Immunohistochemical demonstration of urotensins I and II in the caudal neurosecretory system of the white sturgeon, *Acipenser transmontanus* Richardson. *Biomed Res* 10 (Suppl 3): 329–340
- Oka S, Chiba A, Honma Y (1993) Peptidergic innervation in the caudal neurosecretory system of some fishes as revealed by double immunostaining. *Zool Sci* 10 (Suppl): 138
- Onstott D, Elde R (1986) Immunohistochemical localization of urotensin I/corticotropin-releasing factor, urotensin II, and serotonin immunoreactivities in the caudal spinal cord of nonteleost fishes. *J Comp Neurol* 249: 205–225
- Owada K, Yamada C, Kobayashi H (1985) Immunoreactivity to antisera of urotensin II and corticotropin-releasing factor in the caudal neurosecretory system of the elasmobranchs and the dorsal cells of the lamprey. *Cell Tissue Res* 242: 527–530
- Pearson D, Shiley JE, Clark BR, Geschwind II, Barkley M, Nishioka RS, Bern HA (1980) Urotensin II: a somatostatin-like peptide in the caudal neurosecretory system of fishes. *Proc Natl Acad Sci USA* 77: 5021–5024
- Rawitch AB, Pollock HG, Brodin L (1992) A neuropeptide Y (NPY)-related peptide is present in the river lamprey CNS. *Neurosci Lett* 140: 165–168
- Sano Y (1965) The caudal neurosecretory system. In "Central Regulation of Internal Secretion" Ed by Katuki S, Igaku Shoin, Tokyo, pp 469–522 (in Japanese, German abstract)
- Suess U, Lawrence J, Lederis K (1986) Radioimmunoassay for fish tail neuropeptides. I. Development of assay and measurement of immunoreactive urotensin I in *Catostomus commersoni* brain, pituitary, and plasma. *J Pharmacol Methods* 15: 335–346
- Vecino E, Ekström P (1992) Colocalization of neuropeptide Y (NPY)-like and FMRFamide-like immunoreactivities in the brain of the Atlantic salmon (*Salmo salar*). *Cell Tissue Res* 270: 435–442
- Waugh D, Youson J, Hims SD, Sowe S, Conlon JM (1995) Urotensin II from the river lamprey (*Lampetra fluviatilis*), the sea lamprey (*Petromyzon marinus*), and the paddlefish (*Polyodon spathula*). *Gen Comp Endocrinol* 99: 323–332
- Yulis CR, Lederis K (1986) Extraurophyseal distribution of urotensin II immunoreactive neuronal perikarya and their processes. *Proc Natl Acad Sci USA* 83: 7079–7083
- Yulis CR, Lederis K (1988) Occurrence of an anterior spinal cerebrospinal fluid-contacting, urotensin II neuronal system in various fish species. *Gen Comp Endocrinol* 70: 301–311
- Yulis CR, Garcia ME, Roderiguez EM (1990) The caudal spinal cord of coho salmon (*Oncorhynchus kisutch*): immunocytochemical evidence of a "caudal serotonergic system". *Cell Tissue Res* 259: 543–550

(Received March 10, 1997 / Accepted May 7, 1997)